Synthetic polymer coatings for long-term growth of human embryonic stem cells

Luis G Villa-Diaz[1,2,12], **Himabindu Nandivada**[3,12], **Jun Ding**[1], **Naiara C Nogueira-de-Souza**[1,4], **Paul H Krebsbach**[2,5], **K S O'Shea**[6], **Joerg Lahann**[3,5,7,8], and **Gary D Smith**[1,9,10,11]

[1]Department of Obstetrics and Gynecology, University of Michigan, Ann Arbor, Michigan, USA

[2]Department of Biologic and Materials Science, University of Michigan, Ann Arbor, Michigan, USA

[3]Department of Chemical Engineering, University of Michigan, Ann Arbor, Michigan, USA

[4]Human Biology Research Laboratory, Barretos Cancer Hospital, Sao Paulo, Brazil

[5]Department of Biomedical Engineering, University of Michigan, Ann Arbor, Michigan, USA

[6]Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, Michigan, USA

[7]Department of Materials Science and Engineering, University of Michigan, Ann Arbor, Michigan, USA

[8]Department of Macromolecular Science and Engineering, University of Michigan, Ann Arbor, Michigan, USA

[9]Department of Urology, University of Michigan, Ann Arbor, Michigan, USA

[10]Department of Molecular and Integrated Physiology, University of Michigan, Ann Arbor, Michigan, USA

[11]Department of Reproductive Sciences Program, University of Michigan, Ann Arbor, Michigan, USA

Abstract

We report a fully defined synthetic polymer coating, poly[2-(methacryloyloxy)ethyl dimethyl-(3-sulfopropyl)ammonium hydroxide] (PMEDSAH), which sustains long-term human embryonic stem (hES) cell growth in several different culture media, including commercially available defined media. The development of a standardized, controllable and sustainable culture matrix for hES cells is an essential step in elucidating mechanisms that control hES cell behavior and in optimizing conditions for biomedical applications of hES cells.

Considerable progress has been made in the development of defined hES cell media[1,2]; however, long-term culture still requires use of recombinant extracellular matrix proteins[3] or animal-derived matrices[4], which are sources of variability[5] and xenogeneic contamination[6]. A compositionally defined matrix that supports hES cell expansion in defined media is critical for determining factors that regulate stem cell growth and differentiation, expanding the use of hES cells in biotechnologies and enabling potential clinical applications. Thus far, synthetic polymer matrices have sustained only short-term hES cell propagation[7–10]. Here, we synthesized six polymer coatings by surface-initiated graft polymerization and tested their ability to promote attachment and proliferation of undifferentiated hES cells (Fig. 1a and Supplementary Methods).

First, we examined growth using mouse embryonic fibroblast (MEF)-conditioned media (CM). H9 hES cells were mechanically harvested from cultures grown on MEFs and placed onto dishes coated with polymer or Matrigel. Matrigel supported adhesion and colony formation of >90% of hES cell aggregates, but no attachment was observed on poly[carboxybetaine methacrylate]. hES cells adhered, but spontaneously differentiated, during the first two passages on poly[[2-(methacryloyloxy)ethyl]trimethylammonium chloride], poly[3-sulfopropyl methacrylate], poly[2-hydroxyethyl methacrylate], and poly[poly(ethylene glycol) methyl ether methacrylate] (Fig. 1a). However, PMEDSAH (Fig. 1b for polymer characterization) supported attachment and proliferation of two hES cell lines (BG01 and H9), long-term growth, as well as normal genetic, proteomic and differentiation potential. Throughout 25 passages, H9 hES cells seeded on PMEDSAH expressed characteristic hES cell markers, displayed a normal karyotype and retained pluripotency (Fig. 1c–f and Supplementary Fig. 1). At passage 20, expression levels of the hES cell markers OCT3/4 (91 ± 3%) and SOX2 (92 ± 2%) were similar to levels expressed by cells cultured on Matrigel (Fig. 1c). Microarray analysis revealed similar expression levels of hES cell–specific genes in cells grown on PMEDSAH or Matrigel (Supplementary Table 1). Further validation using qPCR revealed similar RNA expression levels of *NANOG*, *OCT3/4* (also known as *POU5F1*) and *SOX2* (Fig. 1d; primers listed in Supplementary Table 2). The pluripotency of H9 hES cells was confirmed at passage 25 by tri-lineage differentiation in teratomas (Fig. 1f). Taken together, these results demonstrate the ability of PMEDSAH to support long-term culture of undifferentiated, pluripotent hES cells.

Next, we grew BG01 and H9 cells on PMEDSAH-coated dishes in the presence of a xeno-free (free of nonhuman animal products) commercially available human cell–conditioned medium (Supplementary Methods). Throughout 15 passages, BG01 and H9 cells showed similar cell population–doubling times (38 ± 6 h and 37 ± 4 h, respectively) that compared well to values reported for Matrigel[11], expressed hES cell markers, retained normal karyotypes and remained pluripotent (Supplementary Fig. 2). On PMEDSAH, a significantly higher H9 hES cell–aggregate adhesion was observed for all passages using human cell–conditioned medium (86 ± 6%) compared to MEF-conditioned media (15 ± 1%; Fig. 2a). Cell-aggregate adhesion was also significantly higher for H9 cells in human cell–conditioned medium than for BG01 cells cultured under the same conditions (47 ± 5%; Supplementary Fig. 2), suggesting that there may be important biological differences between cell lines in their expression of adhesion receptors.

Finally, we examined the ability of PMEDSAH to support hES cell cultures in two serum-free defined media[1,2,4,12] (StemPro and mTeSR; Supplementary Methods). Cells grown in mTeSR could not be passaged, but StemPro medium was able to support ten passages of H9 hES cells (Fig. 2). BG01 hES cells could not be passaged beyond three passages in StemPro (Supplementary Fig. 4). Throughout ten passages of H9 cells in StemPro medium, cell population–doubling times, expression of hES cell markers and normal karyotypes were

confirmed (Fig. 2a,b). Moreover, H9 cells maintained the ability to differentiate into endoderm, mesoderm and ectoderm (Fig. 2c,d).

The ability of PMEDSAH to support hES cell culture in defined media suggest the utility of this coating for commercial hES cell expansion. Additional studies will be required to determine contribution of the various physicochemical properties of the polymer, such as wettability, mechanical stiffness, surface topography and zeta potential. Compared to animal-derived matrices such as Matrigel,. PMEDSAH is chemically defined, can be synthesized reproducibly and has long-term stability. Unlike natural and recombinant matrices, PMEDSAH-coated dishes can be handled and stored with relative ease. This matrix therefore represents a significant step in the development of a fully defined, reproducible culture system for hES cell expansion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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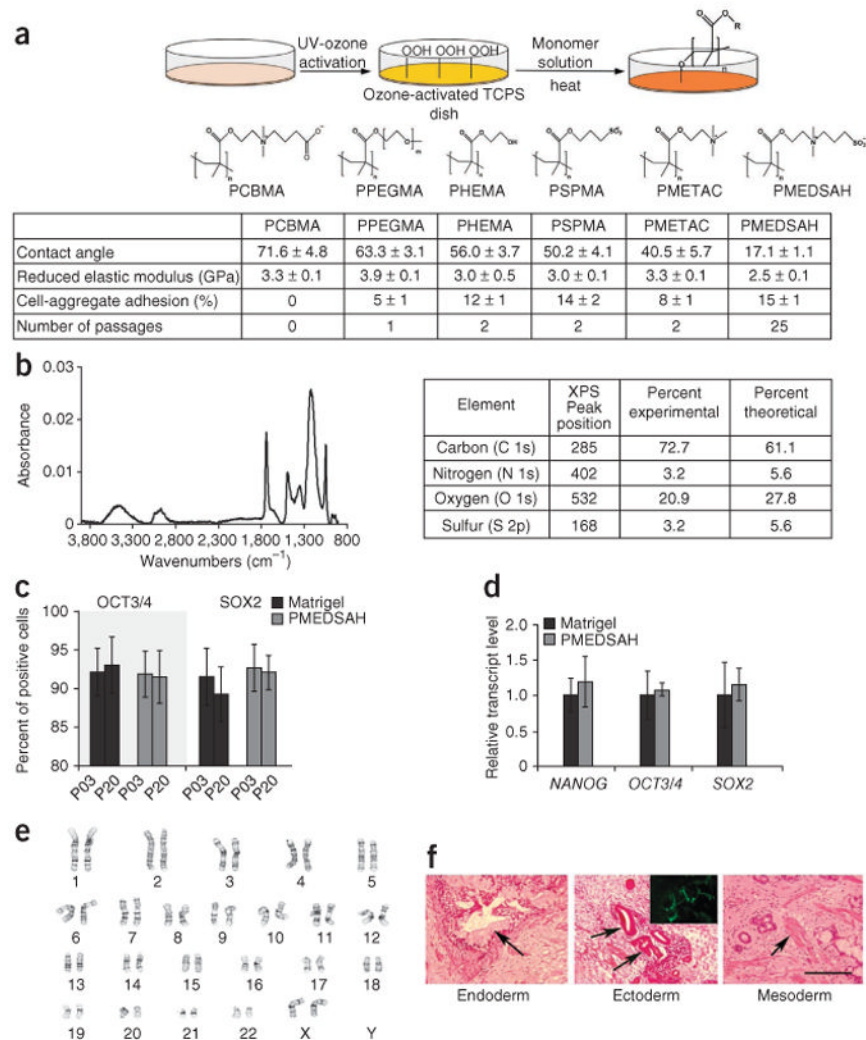12. Prowse AB, et al. Stem Cells Dev. 2009; 18:1135–1140. [PubMed: 19397457]

	PCBMA	PPEGMA	PHEMA	PSPMA	PMETAC	PMEDSAH
Contact angle	71.6 ± 4.8	63.3 ± 3.1	56.0 ± 3.7	50.2 ± 4.1	40.5 ± 5.7	17.1 ± 1.1
Reduced elastic modulus (GPa)	3.3 ± 0.1	3.9 ± 0.1	3.0 ± 0.5	3.0 ± 0.1	3.3 ± 0.1	2.5 ± 0.1
Cell-aggregate adhesion (%)	0	5 ± 1	12 ± 1	14 ± 2	8 ± 1	15 ± 1
Number of passages	0	1	2	2	2	25

Element	XPS Peak position	Percent experimental	Percent theoretical
Carbon (C 1s)	285	72.7	61.1
Nitrogen (N 1s)	402	3.2	5.6
Oxygen (O 1s)	532	20.9	27.8
Sulfur (S 2p)	168	3.2	5.6

Figure 1.

Long-term culture of H9 hES cells on PMEDSAH with MEF-conditioned media. (**a**) Schematic diagram showing graft-polymerization used to synthesize the polymer coatings and their chemical structures. Tissue culture polystyrene dishes were first activated by UV ozone and then methacrylate-based monomers were polymerized from the surface. Table lists contact angle, reduced elastic modulus (GPa) (mean ± s.d.), initial hES cel aggregate adhesion (mean ± standard error) and number of passages achieved on each polymer coating. (**b**) Fourier transform infrared spectrum of PMEDSAH coating showing distinct bands at 1,732.9 cm^{-1} and 1,208.4 cm^{-1}, which indicated presence of carbonyl and sulfonate groups, respectively. Table lists elemental composition of PMEDSAH obtained using X-ray photoelectron spectroscopy. Relative composition of these elements was in agreement with the expected chemical composition of PMEDSAH. (**c**) Percentage (mean ± standard error) of hES cells expressing OCT3/4 and SOX2 at passages 3 (P03) and 20 (P20). (**d**) Relative transcript levels of *NANOG*, *OCT3/4* and *SOX2* from hES cells cultured on PMEDSAH and Matrigel. (**e,f**) After 25 passages, hES cells cultured on PMEDSAH and Matrigel (Supplementary Fig. 3) (**e**) maintained a normal karyotype and (**f**) retained pluripotency as demonstrated by teratoma formation in mmunosuppressed mice. H&E-stained paraffn sections indicating endoderm (goblet-like cells at arrow), ectoderm (neuroepithelial aggregates at arrow; and cells expressing neuron-restricted protein β-III tubulin in inset) and

mesodermal derivatives (cartilage, connective tissue and muscle at arrow). Scale bar, 200 μm.
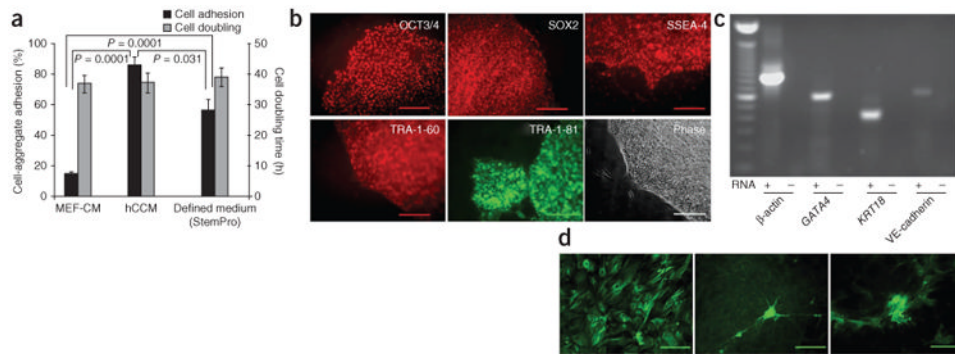
Figure 2.
PMEDSAH supports culture of hES cells in defined medium. (**a**) Percentage (mean ±
standard error) of cell aggregate adhesion (number of aggregates attached with respect to
total aggregates passaged) and population doubling time (twofold increase in colony area)
for H9 hES cells cultured on PMEDSAH in MEF-conditioned medium, human cell–
conditioned medium and defned medium. *P*-values calculated using unpaired *t*-test. (**b**)
Fluorescence micrographs of colonies of H9 cells cultured on PMEDSAH in StemPro
medium showing expression of hES cell markers: OCT3/4, SOX2, SSEA-4, TRA-1-60 and
TRA-1-81; and a phase-contrast image. (**c**) RT-PCR analysis of RNA from embryoid bodies
showing expression of endoderm (*GATA4*), ectoderm (*KRT18*) and mesoderm derivatives
(*VE-cadherin*; also known as *CDH5*). *β-Actin* (also known as *ACTB*) was used as positive
control, and for each primer set tested, a reaction lacking RNA was assessed in parallel as a
negative control. Scale bars, 200 μm, except for SOX2, which is 100 μm (**d**) Micrographs
showing immunoreactivity for α-fetoprotein (endoderm), β-III tubulin (ectoderm) and
smooth muscle actin (mesoderm) demonstrating the pluripotent state of H9 cells cultured on
PMEDSAH in StemPro medium. Scale bars, 200 μm.